EFFECTS OF PALM OIL MILL EFFLUENT ANAEROBIC SLUDGE PRETREATMENT TEMPERATURE ON BIOHYDROGEN PRODUCTION AND THE INOCULUM MICROBIAL COMMUNITY

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ABSTRACT

Biohydrogen production yield from dark fermentation could be improved by inhibiting interspecies hydrogen transfer to methanogens, homoacetogens and suppress non-biohydrogen producers as metabolic competitors. Heat pre-treatment has been extensively used for this purpose. In this study, the effects of heat pre-treatment temperatures on the performance of mesophilic biohydrogen dark fermentation system and the inoculum microbial community were evaluated, using POME anaerobic sludge as inoculum. Heat pre-treatment of the anaerobic sludges were conducted at 50°C, 65°C, 80°C and 100°C for 30 minutes. Biohydrogen production was evaluated under batch fermentation (30°C, initial pH 5.5-6.0, 180 rpm). Shotgun metagenomics analysis was carried out on the raw, untreated and treated sludge inoculum after fermentation. The results showed that pre-treatment of the inoculum at 65°C produced the highest biohydrogen yield of 0.67 mol H_2/mol hexose followed by pre-treatment at 80°C which produced 0.62 mol H_2/mol hexose. Untreated inoculum yielded 0.30 mol H_2/mol hexose while 50°C and 100°C pre-treated inoculum produced less than untreated anaerobic sludge. Methane was not detected in any of the fermentation reactions. Shotgun metagenomics revealed that inoculum heat pre-treatment temperatures influenced the microbial communities in biohydrogen production fermentation. Inoculum pre-treated at 65°C and 80°C were enriched with the most biohydrogen-producing taxa, mainly spore-forming *Clostridia* which generate biohydrogen via butyrate-type fermentation pathways, producing butyric and acetic acids. Untreated anaerobic sludge fermentation was not significantly enriched with biohydrogen-producing microbial taxa. Heat pre-treated inoculum at a lower temperature of 50°C was enriched with non-spore-forming biohydrogen producers from *Klebsiella*, *Escherichia* and *Citrobacter* genera. These genera produced low biohydrogen yield by shifting the fermentation pathway to mixed-acid fermentation, producing a mixture of acetic and butyric acids and ethanol. The presence of non-biohydrogen producers, such as lactic acid bacteria also decreased biohydrogen production of the 50°C pre-treated inoculum, due to the lactic acid and ethanol fermentations. Heat pre-treatment at higher temperature of 100°C selectively enriched spore-forming microbial taxa. Among the species are biohydrogen producers *B. coagulans*, homoacetogens *C. magnum*, and non-biohydrogen producers from *Bacillus* species. These species re-direct the fermentation pathway to mixed-acid fermentation and produced high concentration of acetic acids. Methanogens, e.g. *M. soehngenii* present in the raw anaerobic sludge were suppressed in all the fermentation reactions. Metabolic functions analyses from the metagenome data showed that biohydrogen production potentially upregulate functions related to cellular processes (prokaryotic cell spore formation), carbohydrate metabolisms (sugar alcohols, monosaccharides, sugar acids and carboxylic acids metabolisms) and energy (fermentation and methanogenesis). In conclusion, inoculum heat pre-treatment is essential to enhance biohydrogen production using POME anaerobic sludge as heat pre-treatment enriched the inoculum with biohydrogen producers and suppress activities of methanogens, non-biohydrogen producers and homoacetogens.

ABSTRAK

Penghasilan biohidrogen menerusi penapaian gelap boleh dipertingkat dengan menghentikan penggunaan hidrogen secara interspesies oleh mikrob seperti metanogen, homoasetogen dan mikrob bukan penghasil biohidrogen sebagai pesaing metabolik. Proses pra-rawatan haba telah digunakan secara meluas untuk tujuan ini. Kajian ini telah menyelidiki kesan suhu pra-rawatan haba kepada penghasilan biohidrogen secara penapaian gelap pada julat mesofilik, dan kepada komuniti mikrob dalam inokulum menggunakan sampel enap cemar anaerobik POME sebagai inokulum. Pra-rawatan haba dibuat pada suhu 50°C, 65°C, 80°C dan 100°C selama 30 minit. Penghasilan biohidrogen dinilai melalui penapaian berkelompok (30°C, pH awal 5.5-6.0, 180 rpm). Kemudian, komuniti mikrob dianalisis melalui teknik metagenomik 'shotgun' menggunakan sampel inokulum enap cemar asal, tanpa rawatan dan sampel yang dirawat haba selepas penapaian berlangsung. Hasil kajian menunjukkan inokulum yang dirawat haba pada 65°C menghasilkan biohidrogen tertinggi iaitu 0.67 mol H_2/mol heksosa diikuti oleh pra-rawatan haba pada 80°C yang menghasilkan 0.62 mol H_2/mol heksosa. Inokulum tanpa rawatan menghasilkan 0.30 mol H_2/mol heksosa manakala inokulum yang dirawat pada 50°C dan 100°C menghasilkan biohidrogen lebih rendah daripada sampel inokulum tanpa rawatan. Metana tidak dikesan dalam semua sampel penapaian yang dianalisis. Analisis penjujukan metagenomik 'shotgun' menunjukkan bahawa suhu pra-rawatan haba mempengaruhi komuniti mikrob dalam proses penapaian. Inokulum yang dirawat pada suhu 65°C dan 80°C diperkaya dengan taksa penghasil biohidrogen paling banyak, terutamanya pembentuk spora *Clostridia* yang menghasilkan biohidrogen melalui penapaian jenis butirat, menghasilkan asid butirik dan asetik. Penapaian menggunakan inokulum tanpa rawatan tidak menunjukkan pengkayaan takson mikrob penghasil biohidrogen. Pra-rawatan haba pada suhu rendah 50°C pula diperkaya dengan taksa penghasil biohidrogen daripada kumpulan bukan pembentuk spora, iaitu genera *Klebsiella*, *Escherichia* dan *Citrobacter*. Genera ini menghasilkan biohidrogen lebih rendah, dengan menukar kepada penapaian asid campuran, menghasilkan asid asetik, butirik dan etanol. Kehadiran mikrob bukan penghasil biohidrogen, seperti bakteria asid laktik juga mengurangkan penghasilan biohidrogen oleh inokulum yang dirawat pada 50°C, disebabkan penapaian asid laktik dan etanol. Pra-rawatan haba pada suhu tinggi, 100°C pula memperkaya taksa mikrob yang membentuk spora. Antaranya ialah penghasil biohidrogen *B. coagulans*, homoasetogen *C. magnum* dan mikrob bukan penghasil biohidrogen daripada spesies *Basilus*. Spesies ini mengubah laluan penapaian kepada penapaian asid campuran dan menghasilkan asid asetik yang tinggi. Metanogen, seperti *M. soehngenii* yang wujud dalam enap cemar anaerobik asal, didapati dalam kelimpahan yang rendah dalam semua sampel penapaian yang dianalisis. Analisis fungsi metabolik daripada data metagenom menunjukkan proses penghasilan biohidrogen diiringi oleh peningkatan fungsi berkaitan proses selular (pembentukan sel spora prokariotik), metabolisma karbohidrat (gula alkohol, monosakarida, asid gula dan metabolisma asid karboksilik) dan tenaga (penapaian dan metanogenesis). Kesimpulannya, pra-rawatan haba adalah penting untuk meningkatkan penghasilan biohidrogen menggunakan enap cemar anaerobik POME sebagai inokulum, dengan memperkaya mikrob penghasil biohidrogen, dan menyekat aktiviti metanogen, mikrob bukan penghasil biohidrogen dan homoasetogen.

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LIST OF ABBREVIATIONS

Ac	-	Acid
Acetyl-CoA	-	Acetyl coenzyme A
Fd	-	Ferredoxin
PFOR	-	Pyruvate ferredoxin oxidoreductase
ADMI	-	American Dye Manufacture Index
Ae	-	Aeration
APHA	-	American Public Health Association
PFL	-	Pyruvate formate lyase
NFOR	-	NADH:ferredoxin oxidoreductase
ATP	-	Adenosine triphosphate
B	-	Before
BESA	-	Bromoethanesulfonic acid
BOD	-	Biochemical oxygen demand
CH_3COOH	-	Acetic acid
CO_2	-	Carbon dioxide
CO_3^{2-}	-	Carbonate ion
$CoCl_2.5H_2O$	-	Cobalt(II) chloride pentahydrate
COD	-	Chemical oxygen demand
COGs	-	Clusters of Orthologous Groups of Proteins
CSV	-	Comma separated value
$CuSO_4.5H_2O$	-	Copper(II) sulfate pentahydrate
DAA	-	DIAMOND alignment archive
DNA	-	Deoxyribonucleic acid
DOE	-	Department of Environment
E	-	After
EFB	-	Empty fruit bunches
$FeSO_4.7H_2O$	-	Iron(II) sulfate heptahydrate
FID	-	Flame ionising detector
FiT	-	Feed-in-Tariff
FMAP	-	Functional Mapping and Analysis Pipeline

GC	-	Gas chromatography
H_2	-	Hydrogen
H_2CO_3	-	Carbonic acid
H_2S	-	Hydrogen sulfide
H_2SO_4	-	Sulfuric acid
H_3PO_4	-	Phosphoric acid
HCl	-	Hydrochloric acid
HRT	-	Hydraulic retention time
Ht	-	Heat
IR	-	Ionising radiation
iTOL	-	Interactive Tree of Life
ITS	-	Intergenic transcribed spacers
K_2HPO_4	-	Dipotassium hydrogen phosphate
KEGG	-	Kyoto Encyclopedia of Genes and Genomes
KOs	-	KEGG Orthologues
LA	-	Linoleic acid
LSU	-	Large ribosomal subunit
$MgCl_2.7H_2O$	-	Magnesium chloride heptahydrate
$MnSO_4.6H_2O$	-	Manganese(II) sulfate hexahydrate
MPOB	-	Malaysian Palm Oil Board
NADH	-	Nicotinamide adenine dinucleotide
Na_2CO_3	-	Sodium bicarbonate
$NaHCO_3$	-	Sodium carbonate
$NaNO_2$	-	Sodium nitrite
NaOH	-	Sodium hydroxide
ND	-	No data
NH_4HCO_3	-	Ammonium bicarbonate
NH_4OH	-	Ammonium hydroxide
NKEA	-	National Key Economic Area
PCA	-	Principal component analysis
PICRUSt	-	Phylogenetic Investigations of Communities by Reconstruction of Unobserved State
PNG	-	Portable network graphics

POME	-	Palm oil mill effluent
Protons	-	H^+
PTFE	-	Polytetrafluoroethylene
R1	-	Forward reads
rRNA	-	Ribosomal ribonucleic acid
SCFAs	-	Short chain fatty acids
SEDA	-	Sustainable Energy Development Authority
SMR	-	Steam methane reforming
SREP	-	Small Renewable Energy Power Program
STAMP	-	Statistical Analysis of Metagenome Profiles
TCD	-	Thermal conductivity detector
TN	-	Total nitrogen
TOC	-	Total organic carbon
TP	-	Total phosphate
TS	-	Total solid
TSS	-	Total suspended solid
TSV	-	Tab separated value
TVS	-	Total volatile solid
TVSS	-	Total volatile suspended solid
VS	-	Volatile solid

LIST OF SYMBOLS

%	-	Percentage
°C	-	Degree Celsius
°C/min	-	Degree Celsius/minute
µL	-	Microlitre
µm	-	Micrometre
10X	-	Ten times
atm	-	Atmosphere
bp	-	Base pair
COD/L	-	Chemical oxygen demand per litre
e	-	Constant (2.71828)
g	-	Gravity of Earth
g	-	Gram
ɣ	-	Gamma
g/L	-	Gram per litre
GB	-	Gigabyte
h	-	Hour
H_{max}	-	Maximum biohydrogen production
K	-	Kelvin
kg	-	Kilogram
kHz	-	Kilohertz
L	-	Litre
L atm/mol K	-	Litre atmosphere per mol Kelvin
M	-	Molar
m	-	Metre
mg	-	Milligram
mg/L	-	Milligram per litre
min	-	Minute
MJ/kg	-	Megajoule per kilogram
mL	-	Millilitre

mL H_2/g COD	-	Millilitre hydrogen per gram chemical oxygen demand
mL H_2/g glucose	-	Millilitre hydrogen per gram glucose
mL H_2/g VS	-	Millilitre hydrogen per gram volatile solid
mL H_2/L h	-	Millilitre hydrogen per gram litre hour
mL/h	-	Millilitre per hour
mL/min	-	Millilitre per minute
mm	-	Millimetre
mM	-	Millimolar
mmol	-	Millimol
mmol H_2/g VSS	-	Millimol hydrogen per volatile suspended solids
mol H_2/mol hexose	-	Mol hydrogen per mol hexose
n	-	Number of moles
n	-	Number of sample replicates
nm	-	Nanometre
P	-	Pressure
P	-	Maximum biohydrogen potential
ppm	-	Parts per million
R	-	Ideal gas constant (0.0821 L atm/mol K)
R_m	-	Maximum biohydrogen production rate
rpm	-	Rotation per minute
t	-	Fermentation time
T	-	Temperature
TB	-	Terabyte
V	-	Volume
v/v	-	Volume per volume
W	-	Watt
w/v	-	Weight per volume
λ	-	Lag phase time

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CHAPTER 1

INTRODUCTION

1.1 Problem Background

Fossil fuels are currently the global primary energy sources. In addition to its exhaustible nature, the use of fossil fuels contributed to 89% of global carbon dioxide emissions and deteriorate the environment (Dhakal et al., 2022; Hanif et al., 2019). Dark fermentative biohydrogen production from organic wastes is part of the strategies in moving towards fully circular economy, with simultaneous environmental and economic benefits. Dark fermentation can help in organic wastes treatment, sustainable bioenergy production and decrease greenhouse gas emissions at the same time (Sharma et al., 2020). A study by the World Bank in 2018 estimated that by 2050, the global wastes generation will grow to 3.4 billion tonnes, with more than 50% of the total waste compositions being organic wastes (Kaza et al., 2018). Malaysia for instance, with palm oil industry as among the largest revenue-generating sector in this country, generates a large amount of the palm oil-related organic wastes every year. These include oil palm trunks, oil palm fronds, empty fruit bunches, mesocarp fruit fibres, palm kernel shells and palm oil mill effluent (POME) (Tan and Lim, 2019). The increasing organic wastes generation and stringent environmental standards imposed by the country has increased the need for sustainable and effective utilisation of organic wastes for conversion into value-added products, such as bioenergy. Methane production through anaerobic digestion is currently one of the commonly used technologies for the treatment of organic wastes to generate bioenergy (Uddin et al., 2021). In addition, biohydrogen production through dark fermentation has also garnered attention recently due to its advantages, such as up to three times higher energy yield than hydrocarbon fuels, and cleaner energy carrier since combustion of hydrogen only produces water (Zhang et al., 2020).

Dark fermentation and anaerobic digestion are biological processes mediated by complex microbiomes, consisting of microorganisms with different properties and metabolic functions, working in synergy to convert organic wastes into biogas (Gómez-Camacho et al., 2021). The anaerobic fermentation comprises four main phases, which are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Dzulkarnain et al., 2022). Hydrogen production involves acidogenesis and acetogenesis stages, before the hydrogen produced is consumed in methanogenesis stage for methane production (Hassa et al., 2018). Methanogenesis is undesired and usually inhibited in dark fermentation for biohydrogen production. Both mixed native microbial community and pure cultures have been used as inoculum. The use of native microbial community, particularly from anaerobic sludge offers more practical advantages than pure cultures. Native community contains a diverse taxa performing different functions, offering several advantages such as rapid degradation of a wide range of organic wastes, more resilient to environmental changes, and easier and cheaper to maintain since a strict aseptic condition is not required (Pachapur et al., 2019; Rocca et al., 2019; Nitipan et al., 2014). However, the co-existence of biohydrogen consumers such as methanogens, and non-biohydrogen producers such as homoacetogens, lactic acid bacteria and sulfate-reducing bacteria in the biohydrogen-producing microbiomes make dark fermentation biochemically complex (Dzulkarnain et al., 2022; Łukajtis et al., 2018).

Full understanding of the microbial community structure present, together with their metabolic capabilities and trophic interactions are important for developing a robust dark fermentation system. Unfortunately, these are still the gap in biohydrogen research (Dzulkarnain et al., 2022; Cabrol et al., 2017; Das, 2017). Until now, biohydrogen yield obtained in practice is limited to 32% of the theoretical yield based on the Thauer limit (4 moles of hydrogen per mole glucose when acetate is the co-produced soluble metabolites) (Patel et al., 2018; Thauer et al., 1977). To overcome the bottleneck of dark fermentation, different inoculum pre-treatment methods such as heat, acid, base, ultrasonication, load shock, aeration, freezing and thawing, ultraviolet light and chemical inhibitors has been adopted (Dzulkarnain et al., 2022; Viana et al., 2019; Pendyala et al., 2012; Mohammadi et al., 2011). Heat pre-treatment, among other methods, has been the most widely reported in mesophilic conditions (Dzulkarnain et al., 2022). Heat pre-treatment enriches biohydrogen producers and

suppresses non-biohydrogen producers and biohydrogen consumers. This will re-direct the fermentation pathways towards biohydrogen production through the final step of dark fermentation. Several heat pre-treatment temperatures have been evaluated to enrich biohydrogen producers in anaerobic sludge from different sources. Temperature range of 80°C-100°C is commonly used (Audu et al., 2021; Li et al., 2020; Yang and Wang, 2019a; Pugazhendhi et al., 2017). Higher temperature range of 103°C-150°C have also been used (Choiron et al., 2020; Tanikkul et al., 2019; Moreno-Andrade et al., 2015). The optimum pre-treatment temperature is determined by the type of inoculum source, the native community present, and possibly the substrate used. It is thus imperative to determine the specific optimum temperature for enriching biohydrogen-producing community and suppressing the hydrogen-consuming ones based on the inoculum source and substrate used. No comparative studies have been conducted so far to evaluate the effects of heat pre-treatment temperatures on biohydrogen production using anaerobic sludge from POME.

Several molecular techniques have been used to unravel the complexity of the highly diverse biohydrogen-producing microbiomes to improve the understanding on their influence towards the stability and efficiency of dark fermentation system. The techniques include conventional cultivation-dependent methods, cultivation-independent methods and next generation multi-omics technologies. Research performed on microbial dynamics in dark fermentation systems is still lacking and not as intensively studied as in anaerobic digester for methane generation (Dzulkarnain et al., 2022). This is despite the rapid advancements of molecular tools contributing to the major discoveries of the diversity and structure of biohydrogen-producing microbiomes. The taxonomic and functional annotation of biohydrogen-producing microbiomes are also hampered by the underrepresentation of the genome sequences of biogas-producing microorganisms in reference databases (Hassa et al., 2018). Thus, this research is designed to investigate the effects of heat pre-treatment temperatures on the performance of biohydrogen-producing mesophilic dark fermentation system, based on the microbial community and functional changes.

1.2 Problem Statement

Dark fermentation has been widely used for organic waste conversion for bioenergy production. Biohydrogen reactors often use anaerobic sludge native microbiota from methanogenic anaerobic digester as inoculum due to the presence of hydrolytic fermentative bacteria (Chen et al., 2022; Audu et al., 2021; Choiron et al., 2020; Li et al., 2020). However, the co-existence of biohydrogen consumers and non-biohydrogen producers in the microbiota could lead to substrate conversion via undesired metabolic pathways, producing other intermediates and by-products such as methane, short fatty acids (SCFAs) (acetate, butyrate, propionate, lactate) and solvents (ethanol and butanol) (Kumar et al., 2018). This production of intermediates and by-products could disrupt the stability of dark fermentation system and limit the feasibility of obtaining the maximum biohydrogen yield to only 32% of the theoretical yield, based on the Thauer limit (Patel et al., 2018).

In addition to the control of operational condition during dark fermentation, inoculum pre-treatment is often performed to improve the biohydrogen yield in mesophilic dark fermentation system. Heat pre-treatment is the simplest method frequently used to enrich biohydrogen producers and suppress biohydrogen consumers and non-biohydrogen producers (Dzulkarnain et al., 2022). Biohydrogen producers such as *Clostridia* and *Bacillus* are spore formers which have high resistant towards extreme environment, whereas biohydrogen consumers and non-biohydrogen producers are mostly non-spore formers and more vulnerable to the extreme environment (Rafieenia et al., 2018). However, heat pre-treatment does not exclusively select for biohydrogen producers. *Enterobacter*, *Klebsiella*, *Ethanoligenens*, *Citrobacter* and *Escherichia* genera are non-spore-forming biohydrogen producers and are easily destroyed under thermal stress. Heat pre-treatment of various inoculum sources including POME anaerobic sludge are frequently performed at one specific temperature mostly at 80°C-100°C, since the undesired microorganisms are effectively suppressed at this high temperature range (Akhbari et al., 2021; Audu et al., 2021; Li et al., 2020; Yang and Wang, 2019a; Pugazhendhi et al., 2017). So far, no comparative study has been conducted to evaluate the effects of heat pre-treatment temperatures on biohydrogen production using anaerobic sludge from POME as inoculum.

1.3 Research Objectives

The objectives of this research were:

1. To evaluate the effects of heat pre-treatment temperature on the biohydrogen production using POME anaerobic sludge as inoculum.
2. To investigate the effects of heat pre-treatment temperature on the dark fermentation inocula based on pH, microbial growth, total carbohydrates and soluble metabolites.
3. To analyse the microbial community dynamics and the metabolic functional changes after heat pre-treatment based on the shotgun metagenomic sequencing data.

1.4 Scope of Research

This research involved anaerobic sludge sample collection from a methanogenic anaerobic digester used for POME treatment at Felda Maokil Palm Oil Mill, Johor, Malaysia. The anaerobic sludge was subjected to physicochemical characterisation according to the procedure of the Standard Methods for Examination of Water and Wastewater by the American Public Health Association (APHA) and the Department of Environment (DOE) (M) Alternative 1995 Standard Methods. Next, the anaerobic sludge samples were heat pre-treated at 50°C, 65°C, 80°C and 100°C, respectively to enrich the biohydrogen-producing microbial communities and suppress the biohydrogen consumers. Anaerobic sludge without heat pre-treatment was used as control. The pre-treated and non-pre-treated anaerobic sludges were then used as inoculum for biohydrogen production through mesophilic dark fermentation. Effects of heat pre-treatment temperatures on the enrichment of biohydrogen-producing microbial communities was evaluated based on biohydrogen, biogas and SCFAs production, pH, microbial growth and total carbohydrate profiles. Metagenomic DNA from raw, pre-treated anaerobic sludge inocula before and after dark fermentation were extracted and sequenced using shotgun sequencing approach. Microbiome sequencing

outputs were processed and analysed using DIAMOND+MEGAN microbiome analysis pipeline to obtain output files in DIAMOND alignment archive (DAA) format. The DAA files were interactively explored, analysed and visualised using several bioinformatic tools to determine the microbial community dynamics and functional changes in the raw and pre-treated anaerobic sludges.

1.5 Significance of Research

This research aims to contribute to a more efficient and robust dark fermentation system for biohydrogen production, from the perspectives of molecular microbiology. Dark fermentative biohydrogen production is a system mediated by complex microbial communities. Knowledge on the taxonomic and functional compositions of biohydrogen reactor, and syntrophic interaction among the members of distinct trophic levels after different inoculum pre-treatment may provide a better understanding on the influence of microbiomes present in the reactor to the entire dark fermentation process. This will ease system scale up, kinetic control and application for the treatment of organic wastes and biohydrogen production using rich waste sources such as POME. Metagenomic data from this research may extend the information on the bacterial and archaeal diversity in biogas-producing microbiomes, specifically the microbiomes underpinning biohydrogen reactors. This research also provides the opportunity to discover an efficient culture system and novel metabolic pathways towards the engineering of a desired microbial community towards maximising biohydrogen production yield and rate.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Dzulkarnain, E. L. N.**, Audu, J. O., Wan Dagang, W. R. Z. and Abdul-Wahab, M. F. (2022). Microbiomes of biohydrogen production from dark fermentation of industrial wastes: current trends, advanced tools and future outlook. *Bioresources and Bioprocessing*, 9(1), 16. https://doi.org/10.1186/s40643-022-00504-8. **(Q2, IF:4.578)**

Non-indexed Book Chapter

1. Audu, J. O., **Dzulkarnain, E. L. N.**, Ibrahim, Z., Ibrahim, N. and Abdul-Wahab, M. F. (2020). *Dark fermentation and bioelectrochemical systems for enhanced biohydrogen production from palm oil mill effluent: current progress, potentials, and future perspectives*, in Zakaria, Z. A., Boopathy, R. and Dib, J. R. (eds.) *Valorisation of Agro-industrial Residues – Volume I: Biological Approaches*. Cham: Springer, pp. 1-35. https://doi.org/10.1007/978-3-030-39137-9_1.